PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 09.08.2000
Applicant's or agent's file reference GWS/DC/20535	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01450	07/05/1999	11/05/1998

Applicant
GEMINI RESEARCH LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Digiusto, M Tel.+49 89 2399-8162

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
GWS/DC/20535		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01450	07/05/1999	11/05/1998
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
GEMINI RESEARCH LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/12/1999	09.08.2000
Name and mailing address of the international preliminary examining authority:	Authorized officer
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Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01450

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-14	as received on	19/05/2000	with letter of	18/05/2000

Claims, No.:

1-14	as received on	19/05/2000	with letter of	18/05/2000

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 2, 5-7, 12-14
	No:	Claims 1, 3, 4, 8-11
Inventive step (IS)	Yes:	Claims 2, 13
	No:	Claims 1, 3-12, 14
Industrial applicability (IA)	Yes:	Claims 1-14
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

The following documents (D) are referred to in this communication; the numbering will be adhered to the rest of the procedure:

D1: WO 97 38130 A (MEDICAL RES COUNCIL ;XU WEIMING (GB)) 16 October 1997 (1997-10-16)

D2: WO 98 08516 A (ISHIHARA TAKAFUMI ;YOSHIKAWA JUNICHI (JP); OKAMURA MIKIO (JP); SUN) 5 March 1998 (1998-03-05)

D3: STEPANOV ET AL.: 'Endothelial NOS gene polymorphism in essential hypertension' EUROPEAN J. HUMAN GENETICS, vol. 6, no. S1, 1998, page 178 XP002113710 & 30th annual meeting of the European Society of Human Genetics, 10-13/5/98

D4: UWABO ET AL.: 'Association of VNTR in the NOS gene with essential hypertension' AM. J. HYPERTENSION, vol. 11, January 1998 (1998-01), pages 125-128, XP002113711

D5: LACOLLEY ET AL.: 'NOS gene polymorphims, blood pressure and aortic stiffness in hypertensive subjects' J. HYPERTENSION, vol. 16, no. 1, January 1998 (1998-01), pages 31-35, XP002113712

D6: TAKAHASHI ET AL.: 'Association analysis of TG repeat polymorphism of the NOS gene with essential hypertension' CLIN. GENET., vol. 52, no. 2, August 1997 (1997-08), pages 83-85, XP002113713

D7: TUNNY ET AL.: 'Association study of the 5' flanking regions of the eNOS gene in POAG' CLIN. EXP. PHARMACOL. PHYSIOL., vol. 25, no. 1, January 1998 (1998-01), pages 26-29, XP002113714

SECTION I

1. The amendments filed with the letter of 18.05.2000 meet the requirements of Article 34(2)(b) PCT.

SECTION V

2. Novelty (Article 33(2) PCT)

2.1 Claims 1, 3, 4 and 8-11 are not novel.

a) Claims 1, 3, 4 and 8-11 are anticipated by D1. D1 (abstract; page 2, second

paragraph; page 3, second and third paragraph; page 4, third paragraph; example 1; page 8) describes that a polymorphic DNA marker based on a pentanucleic repeat has been identified in the iNOS gene. The repeat is located approximately 2.8kb upstream the promoter region of the iNOS gene ("a risk polymorphism is present of in the promoter of an inducible nitric oxide synthase gene" according to present claim 1) and it is implicated in certain common human diseases such as hypertension and the Alzheimer disease ("A method for diagnosing hypertension or a predisposition to hypertension" according to present claim 1). The number of repeats has been identified by PCR amplification, i.e. implicitly this means the use of primers ("one or more PCR primers" according to present claim 8), gel electrophoresis and sequence analysis ("reference markers", "reference gel", "reference chart" according to present claims 9-11).

b) Claim 1 differs from D2 to D7 in that claim 1 describes a method for diagnosing a hypertension determining whether a risk polymorphism is present in the promoter of an inducible nitric oxide synthase.

2.2 Claims 2, 5-7, 12-14 are novel.

Claims 5, 6 and 7 relating, to a method of predicting response to hypertension therapy (claim 5), a method of diagnosing hypertension (claim 7), a method for diagnosing syndrome X (claims 12), methods of diagnosing a disease comprising linkage equilibrium (claims 6 and 14), are not disclosed in the prior art documents.

Claims 2 and 13, relating to a method for diagnosing a disease, wherein the risk polymorphism is a four base pair insertion, is not disclosed in the prior art documents.

3. Inventive Step (Article 33(3) PCT)

3.1 Claims 5-7, 12 and 14 do not involve an inventive step

D1 is considered to be the closest prior art.

Claim 5 differs from D1 in that claim 5 describes a method of predicting response

to hypertension therapy, comprising diagnosing the genotype of a iNOS gene.

Claims 6 and 14 differ from D1 in that claims 6 and 14 describe methods of diagnosing hypertension (claim 6) or Syndrome X (claim 14) or predisposition of hypertension (claim 6) or Syndrome X (claim 14) comprising screening the whole of or a part of an iNOS gene for a polymorphism in linkage equilibrium with a polymorphism in or near the promoter region of an iNOS gene.

Claim 7 differs from D1 in that claim 7 describes a method of locating a further polymorphism correlated with a known polymorphism in or near the promoter region of an iNOS gene comprising locating a further polymorphism and correlating it with the known iNOS gene polymorphism.

Claim 12 differs from D1 in that claim 12 describes a method for diagnosing Syndrome X or a predisposition to Syndrome X comprising determining whether a risk polymorphism is present in the promoter of the inducible nitric oxide synthase gene.

The technical problem to be solved would appear to reside in finding an alternative method based on the genotyping of the iNOS gene.

The skilled person, equipped with the knowledge of D1 (page 2, second paragraph) that a pentanucleotide repeat in the iNOS gene is useful as a micro satellite marker in disease diagnosis, would be motivated to apply this marker in methods as described in claims 5-7, 12 and 14, since such methods are developed as soon as a useful marker is known.

3.2 Claims 2 and 13 would appear to involve an inventive step.

Claims 2 and 13, relating to methods for diagnosing a disease or a predisposition to a disease wherein the risk polymorphism is a four base pair insertion located between position -891 and -575 5' to the transcriptional start site, is neither disclosed nor suggested in the prior art documents. Thus, the skilled person would not be motivated to select this four base pair insertion as marker in a method of diagnosing a disease.

SECTION VII

4. The phrase "and incorporated by reference..." as mentioned e.g. on page 5 (line 4) contravenes the requirement that the application needs to be self contained (see further Guidelines C-II 4.17).

SECTION VIII

5. The term "or a contributory component thereof" in claims 7, 8 is not clear and contravenes Article 6 PCT.

Claims:

1. A method for diagnosing hypertension or a predisposition to hypertension comprising determining whether a risk polymorphism is present in the promoter of an inducible nitric oxide synthase (iNOS) gene.

2. A method according to claim 1, wherein the risk polymorphism is a four base pair insertion located between positions -891 and -575 5' to the transcription start site in the promoter of the iNOS gene.

3. A method according to claims 1 and 2 comprising determining whether an individual is homozygous or heterozygous for a risk polymorphism in a NOS gene.

4. A method of diagnosis and treatment of hypertension comprising diagnosing hypertension or predisposition thereto according to any previous claim, and treating an individual to reduce, prevent or otherwise ameliorate hypertension.

5. A method of predicting response to hypertension therapy, comprising diagnosing genotype of an iNOS gene.

6. A method of diagnosing hypertension or predisposition to hypertension comprising screening the whole of or a part of an iNOS gene for a polymorphism in linkage disequilibrium with a polymorphism in or near the promoter region of an iNOS gene.

7. A method of locating a further polymorphism correlated with a known polymorphism in or near the promoter region of an iNOS gene comprising;

(a) locating a further polymorphism and correlating it with the known NOS gene polymorphism; and

(b) testing whether the further polymorphism is linked to hypertension or any contributory component thereof.

8. A kit for diagnosis of predisposition or susceptibility to hypertension comprising:-

(a) one or more PCR primers for determining genotype of the promoter region of an iNOS gene; and

(b) apparatus for correlating iNOS promoter genotype with risk of predisposition or susceptibility to hypertension or any contributory component thereof.

9. A kit according to claim 8, wherein said apparatus comprises a set of reference markers.

10. A kit according to claim 14, wherein said apparatus comprises a reference gel.

11. A kit according to claim 14, wherein said apparatus comprises a reference chart.

12. A method for diagnosing Syndrome X or a predisposition to Syndrome X comprising determining whether a risk polymorphism is present in the promoter of an inducible nitric oxide synthase (iNOS) gene.

13. A method according to claim 12, wherein the risk polymorphism is a four base pair insertion located between positions -891 and -575 5' to the transcription start site in the promoter of the iNOS gene.

14. A method of diagnosing Syndrome X or predisposition to Syndrome X comprising screening the whole of or a part of an iNOS gene for a polymorphism in linkage disequilibrium with a polymorphism in or near the promoter region of an iNOS gene.